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Tissue Damage and Nutritional Factors in Experimental Respiratory Tract (Co-)Carcinogenesis

by P. G. J. Reuzel,* V. J. Feron,* B. J. Spit,* R. B. Beems* and R. Kroes*

Cofactors involved in respiratory tract carcinogenesis were studied in Syrian golden hamsters or in rats using benzo(a)pyrene as the carcinogenic agent. These factors included severe tissue damage induced by electro-coagulation, glass fibers administered by intratracheal instillation, acetaldehyde as irritant vapor, food restriction, and nutrients such as vitamin A and saturated and unsaturated fats. In addition, the effects of a combined exposure to four different major gaseous cigarette smoke components—methyl nitrate, isoprene, methyl chloride and acetaldehyde—and to one solid cigarette smoke component—norharman—were examined in short- and long-term inhalation studies. An interesting finding was the carcinogenicity of acetaldehyde, of which the possible mechanism is briefly discussed. Another conspicuous observation was the substantial increase in number and size of lipid droplets in alveolar fibroblasts of hamsters fed a high vitamin A diet.

Introduction

The respiratory tract is an organ system which is continuously in contact with the environment for 24 hr each day. During each respiration, small quantities of natural or artificial contaminants present in the surrounding air are passed through this organ system. There they come into contact with the large (in man 30 m^2 or more) highly active area of the internal surface of the respiratory tract. Depending on time, concentration and on chemical and physical properties, the contaminants may exert effects that result in the formation of tumors. Lung cancer as a result of tobacco smoking is a clear example. Though it is known that several components of cigarette smoke have carcinogenic properties (e.g., acetamide, acrylonitril, polycyclic aromatic hydrocarbons and nitrosamines) (*1*), the high incidence of lung cancer in man as a result of smoking habits is not yet fully understood. It has been suggested that unidentified noncarcinogenic components in cigarette smoke or air contaminants from other sources may play a modulating role in the formation of lung cancer. It is also believed that the occurrence of lung cancer can not only be explained by exposure to air contaminants, but that other external factors, such as nutrition, may also be of importance.

Epidemiological studies suggest a relationship between nutritional factors and various types of cancer, including lung cancer. In experimental animals, there is a correlation between the formation of respiratory tract tumors and caloric restriction (*2*) or vitamin A supply (*3-5*). These modifying factors may influence the development and progression of tumors considerably. In this presentation an overview is given of studies currently being carried out at Institute CIVO-Toxicology and Nutrition TNO. These studies are undertaken in efforts to elucidate the potential importance of promoters, cocarcinogens, and initiators in the etiology of lung cancer. In this respect it is reasonable to define what is meant by the term "carcinogen" and to discuss the mode of action of carcinogens.

Definition and Mode of Action of Carcinogens

A carcinogen can be defined as a compound which is capable of inducing tumors. Thus, the criterion for carcinogenicity of a substance is the biological end effect, i.e., cancer. However in assessing

*Institute CIVO-Toxicology and Nutrition TNO, P.O. Box 360, 3700 AJ ZEIST, The Netherlands

the carcinogenic potential of a chemical, consideration should be given to the mechanism by which the compound induced the carcinogenic effect (*6*). On the basis of their mode of action two categories of carcinogenic substances can be distinguished (*7*): genotoxic carcinogens which act directly on the genetic material, thereby inducing an irreversible, self-replicating effect (imprint, memory effect) and are termed complete carcinogens, and carcinogens acting by mechanisms which differ from these ones; such carcinogens include substances that disturb hormonal balance, act according to the principle of nonspecific stimulation (e.g. "solid state" carcinogenesis), induce microsomal enzymes or bring about suppression or overstimulation of the immune apparatus.

Since the mechanisms of carcinogen-induced cancers may be very complicated and involved both genetic and/or nongenetic events, information on the mode of action of carcinogens may be difficult to obtain. However, elucidation of the mode of action of carcinogens is of importance, since results to man depends on the knowledge of the mode of action of a carcinogenic substance (*6-8*).

Animal Studies

Studies undertaken to identify promoting factors in respiratory carcinogenesis are less frequently performed as those to identify initiators of lung cancer. In our institute the following factors are being studied: local damage, irritation by glass fibers or gases and nutritional modulation. An overview of the experimental design and, if available, preliminary results of these studies with hamsters and rats will be given. In addition, the possible mechanism of action of the cancer-inducing agent, acetaldehyde, will be discussed.

Significance of Local Damage of the Respiratory Tract for the Induction of Tumors at the Site of Damage

The presence of metaplastic squamous epithelium in the bronchi of smokers is generally regarded as a predisposition for the formation of a tumor. After severe local damage of the tracheal or bronchial mucous membranes, this tissue will partly be replaced by scar tissue. It has been suggested that this scar tissue is more sensitive for malignant cell transformation as compared to original tissue. To investigate this hypothesis studies were carried out with hamsters in which the respiratory tract is severely damaged at a given site and which are intratracheally treated with benzo(a)pyrene (B(a)P).

Local Damage of the Tracheal Mucous Membrane by Electrocoagulation

In order to be able to induce a certain damage by electrocoagulation, it was necessary to develop a method by which such damage could be induced in a standardized way. During a preliminary study with hamsters, the degree of the damage of the trachea, the repair of the damaged tissue and the variation of it between the individual hamsters were studied. Coagulation resulted in necrosis of the epithelium and the underlying connective tissue, the cartilage included. Signs of inflammation (hyperemia, edema and a slight infiltrate of polymorphnuclear leukocytes) were already visible after 2 hr; after 8 hr this effect was more prominent (Fig. 1 and 2). After 24 hr, severe inflammation, granulation tissue and flattened epithelial cells partly covering the damaged area were visible. After 3 and 7 days the lesions were invariably covered with stratified squamous or cuboidal epithelium often containing large nuclei. An interesting finding was the necrotic cartilage being removed by chondroclasts. After 14 and 21 days the epithelium varied from almost normal ciliated epithelium to stratified squamous epithelium with papilliferous projections. Removal of necrotic cartilage was accompanied by deposition of new regenerated cartilage (Fig. 3). It was clear that after a recovery period of 21 days the local damage had not yet been fully restored.

Effects of Intratracheal Administration of Glass Fibers with or without B(a)P

The biological activity of glass fibers depends on length and diameter of the fibers. Intrapleurally or intraperitoneally applied glass fibers are capable of inducing mesotheliomas (*9, 10*). However, inhalation of glass dust by several rodent species did not result in evidence for carcinogenic properties of the glass dust used (*11*).

Large fibers are able to damage the alveolar wall. This could be shown in a time-sequence study with hamsters, which received intratracheally one single dose of 10 mg glass fibers suspended in saline. The response of the pulmonary tissue is studied by means of light and electron microscopy at various points of times after treatment. Acute inflammatory reaction (granulocytes and macrophages) and necrosis were seen during the first 24 hr after treatment (Fig. 4). By 3 days post-treatment the acute inflammation had largely disappeared; now there were many alveolar macrophages laden with glass fibers, and alveolar septa were thickened and often lined by cuboidal epithelial cells (Fig. 5). After 3 and 6 months clusters of free glass fibers were still visi-

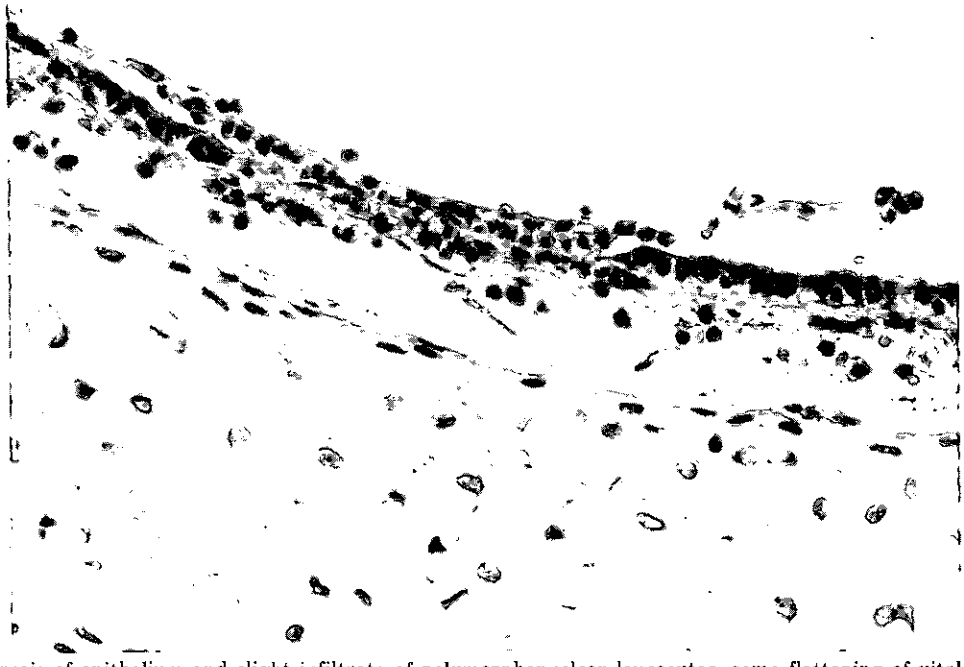

FIGURE 1. Necrosis of epithelium and slight infiltrate of polymorphonuclear leucocytes; some flattening of vital epithelial cells. Trachea, hamster, 2 hr after coagulation. H&E, × 400.

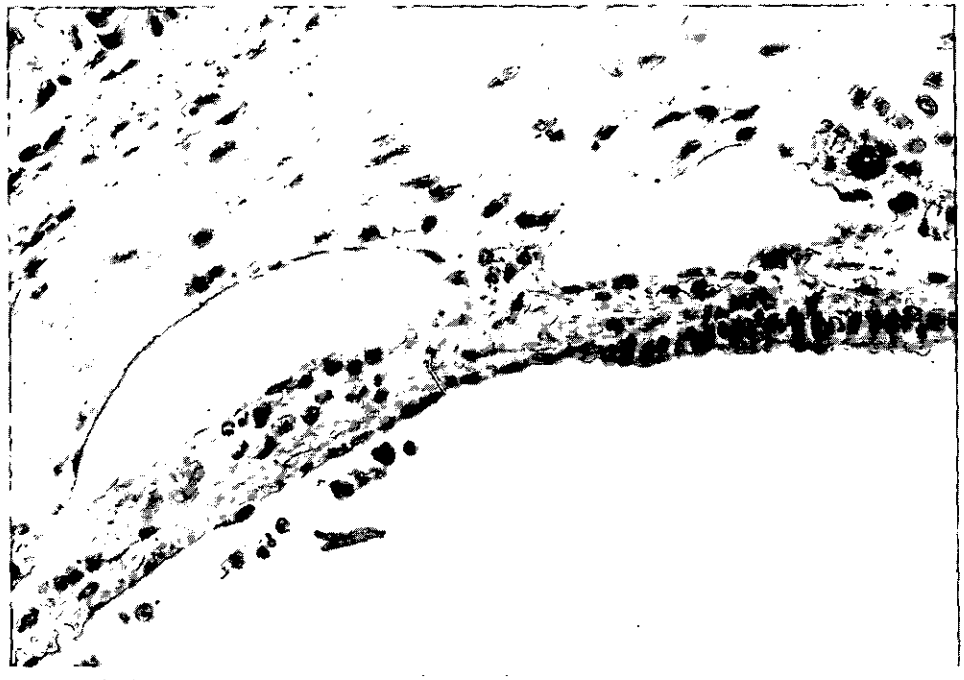

FIGURE 2. Flattened epithelial cells which overgrow the necrotic area. Trachea, hamster, 8 hr after coagulation. H&E, × 400.

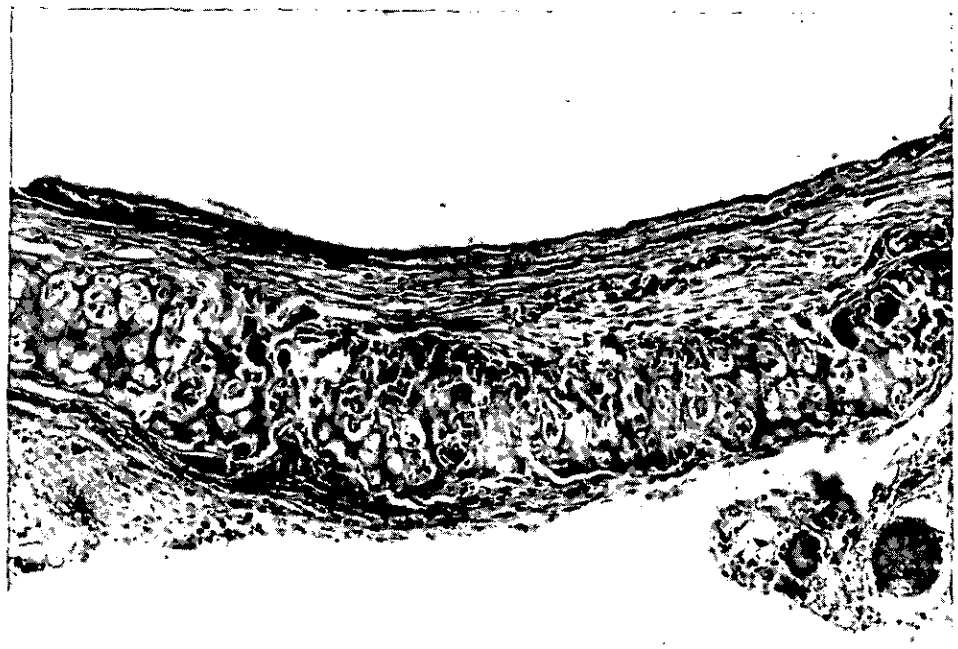

FIGURE 3. Flattened epithelium with irregular nuclei, granulation tissue and cartilage being removed by chondroclasts. Trachea, hamster, 14 days after coagulation. H&E, × 160.

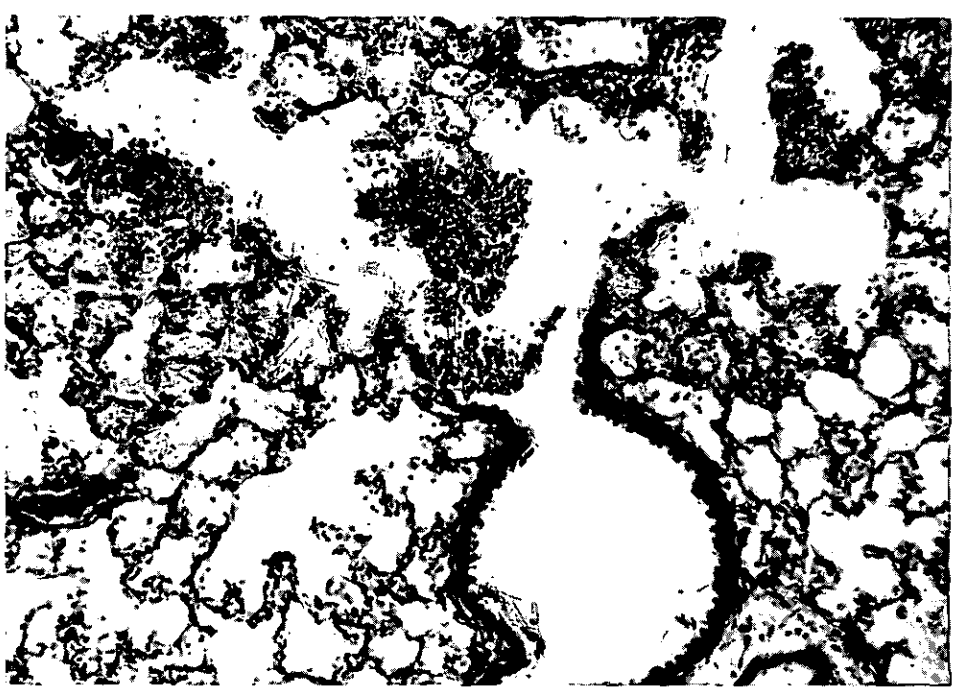

FIGURE 4. Many glass fibers in bronchiolus and alveoli; severe granulocytic inflammatory reaction. Lungs, hamster, 8 hr after intratracheal administration of glass fibers. H&E, × 160.

FIGURE 5. Many macrophages filled with glass fibers and thickened alveolar septa. Lungs, hamsters, 3 days after intratracheal administration of glass fibers. H&E, × 400.

ble, and many macrophages with glass fibers were not only present in alveoli but also in regional lymph nodes; bronchiolar alveoli were often lined by cuboidal epithelium. Results of the ultrastructural studies are not yet available.

In a long-term study with hamsters glass fibers with or without B(a)P are given intratracheally once every fortnight for a period of 52 weeks; hamsters similarly treated with crocidolite asbestos with or without B(a)P serve as controls. Six groups of hamsters, each consisting of 35 males and 35 females, are treated as follows: group 1 (control): 0.2 mL saline; group 2: 1 mg B(a)P in 0.2 mL saline; group 3: 1 mg glass fibers in 0.2 mL saline; group 4: 1 mg glass fibers + 1 mg B(a)P in 0.2 mL saline; group 5: 1 mg crocidolite in 0.2 mL saline; group 6: 1 mg crocidolite + 1 mg B(a)P in 0.2 mL saline. This study has progressed for 9 months.

Chronic Inhalation Study of Acetaldehyde

Acetaldehyde is one of the major components in cigarette smoke. Levels of more than 200 ppm are formed in the gaseous phase of cigarette smoke (*1*).

A long-term inhalation study in Syrian golden hamsters showed that acetaldehyde at a level of 1500 ppm is capable of inducing inflammatory changes and severe hyperplasia and metaplasia of the epithelium in the upper segments of the respiratory tract; no tumors attributable to acetaldehyde were found, but there was some evidence of acetaldehyde enhancing the development of B(a)P-initiated tracheal tumors (*12*). A second similar experiment with a high concentration of acetaldehyde (2500/1650 ppm) revealed that again severe hyperplasia and metaplasia occurred in the upper segments of respiratory tract, but now also a few carcinomas were found in the nose and larynx (Table 1). The cocarcinogenic properties of this high level of acetaldehyde is evidenced by a higher incidence of B(a)P-induced tracheo-bronchial carcinomas (*13*). Recent studies have shown that acetaldehyde may induce genetic damage (*14*). If, indeed, such genetic effects of acetaldehyde were involved in the induction of respiratory tract tumors, as seen in the present study, acetaldehyde should be considered a genotoxic carcinogen. On the other hand, the strong cytotoxic activity of acetaldehyde is undoubtedly responsible for the hyperplasia, metaplasia and inflammation in the upper airways seen in acetaldehyde-exposed hamsters. Since this process of recurrent tissue damage and repair may possess "promoting" properties, the carcinogenicity of acetaldehyde may be based on nonspecific stimulation of relevant genetic damage caused either by background or endogenous genotoxins. For the time being, it seems justifiable to consider acetaldehyde a carcinogen with a weak initiating and a strong "promoting"

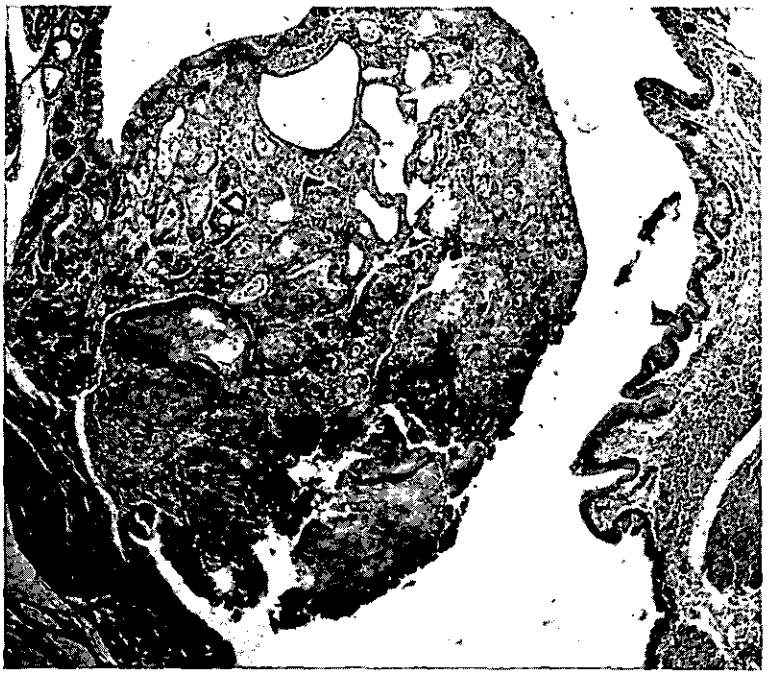

FIGURE 6. Adenosquamous carcinoma of the larynx. Hamster, acetaldehyde, week 78. H&E, × 43.

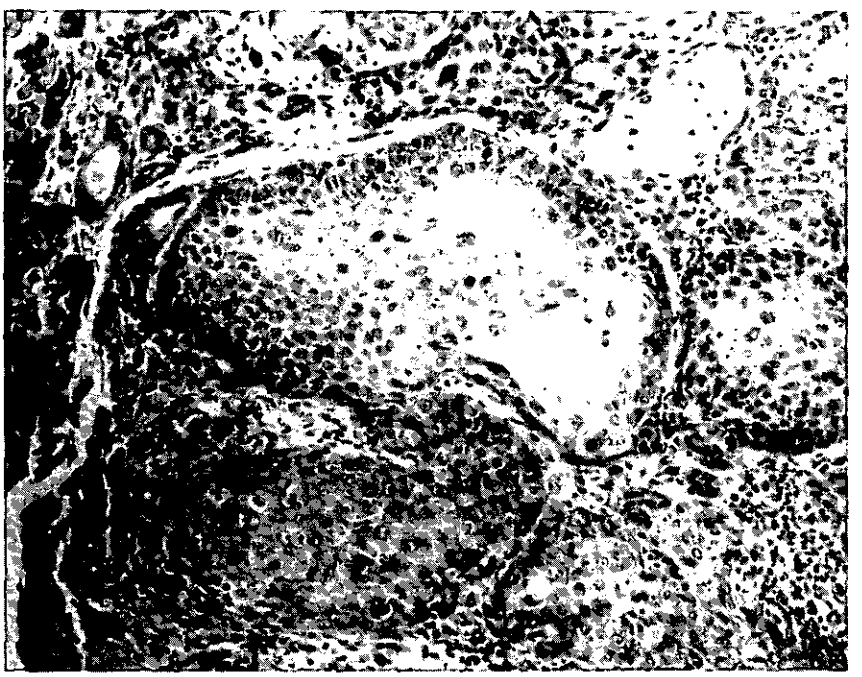

FIGURE 7. Detail of the tumor depicted in Fig. 6. Note the adenosquamous pattern and the infiltrative growth. H&E, × 170.

Table 1. Type and incidence of tumors in the nose, larynx and tracheobronchial tree of hamsters exposed to air or acetaldehyde vapor and treated intratracheally with B(a)P.[a]

Type of tumor	Sex	Incidence of tumors					
		Inhalation of air			Inhalation of acetaldehyde		
		Saline[b]	B(a)P (18.2 mg)[c]	B(a)P (36.4 mg)[d]	Saline[b]	B(a)P (18.2 mg)[c]	B(a)P (36.4 mg)[d]
Nose		(n = 24)	(n = 28)	(n = 30)	(n = 27)	(n = 28)	(n = 27)
Papilloma/adenoma	M	0	0	0	1	0	0
Carcinoma	M	0	0	0	1	2	1
Larynx		(n = 20)	(n = 28)	(n = 29)	(n = 23)	(n = 26)	(n = 25)
Papilloma	M	0	0	1	1	1	1
Carcinoma	M	0	0	0	5	7	8
Trachea/bronchi		(n = 30)	(n = 29)	(n = 29)	(n = 28)	(n = 28)	(n = 27)
Papilloma	M	0	3	7	0	3	2
Carcinoma	M	0	0	3	0	1	15
Sarcoma	M	0	0	1	0	0	2
Nose		(n = 23)	(n = 27)	(n = 24)	(n = 26)	(n = 28)	(n = 22)
Papilloma	F	0	0	0	0	0	0
Carcinoma	F	0	0	0	1	1	0
Larynx		(n = 22)	(n = 27)	(n = 24)	(n = 20)	(n = 23)	(n = 23)
Papilloma	F	0	1	0	1	2	1
Carcinoma	F	0	0	0	3	5	3
Trachea/bronchi		(n = 28)	(n = 27)	(n = 24)	(n = 29)	(n = 29)	(n = 29)
Papilloma	F	0		1	0	3	2
Carcinoma	F	0	0	3	0	1	10

[a]The number of animals n in each group is given in parentheses.
[b]Given intratracheally (0.2 mL), weekly for 52 weeks.
[c]Given intratracheally in 52 weekly doses of 0.35 mg.
[d]Given intratracheally in 52 weekly doses of 0.70 mg.

(cocarcinogenic) activity. The latter activity most probably only occurs at levels exerting cytotoxicity. One might speculate that the ability of noncytotoxic levels of acetaldehyde to induce tumors is very close to zero.

To verify the findings with acetaldehyde in hamsters and to increase their relevance for humans, it was decided to examine the toxicity and carcinogenicity of this compound in another animal species. Therefore, short- and long-term inhalation studies with acetaldehyde in rats have meanwhile been initiated. In a 4-week repeated exposure study with rats degenerative changes with or without various degrees of hyperplasia and metaplasia of the nasal epithelium were found at levels of 400 ppm and higher (up to 5000 ppm); in the larynx and trachea epithelial alterations only occurred at levels of 2200 and 5000 ppm. The respiratory tract changes showed a clear positive dose-response relationship. A long-term rat study with an interim kill design using exposure levels of 0, 750, 1500 and 3000/2000 ppm acetaldehyde and 105 males and 105 females in each group had been in progress for 7 months as of October 1981. The aforementioned findings with acetaldehyde seem of particular importance in the light of recent data on the induction of squamous cell carcinomas in the nose of rats by exposure to formaldehyde vapor (*15*).

It seems not illogical to assume that the mode of action of formaldehyde with respect to its carcinogenicity corresponds to that of acetaldehyde. The implication would be that the carcinogenic risk from exposure to noncytotoxic levels of formaldehyde is practically negligible.

Nutrition as Cofactor in Respiratory Tract Carcinogenesis

Epidemiological research suggests that nutritional habits may be of importance for the occurrence of various types of cancer, such as breast cancer, prostatic cancer and colon cancer (*16-19*). Tobacco smoking is an important causative factor for lung cancer; to assume a relation between diet and lung cancer seems to be rather peculiar. This might be the reason that in the literature very little attention has been paid to a possible role of the diet in the etiology and therapy of lung cancer. However, Carrol and Kohr (*17*) found a positive correlation between high dietary fat consumption and lung cancer in males, and Schrauzer (*18*) observed a positive correlation in females. In addition, the latter author observed a positive correlation between lung cancer and the consumption of sugar in males and between lung cancer and the consumption of meat and eggs in females. The correlations mentioned above are supported by the results of some experiments in

laboratory animals. In mice the incidence of spontaneous lung tumors was decreased by caloric restriction (*2*).

Furthermore high levels of vitamin A reduced the number of induced lung tumors in rats (*3*) and hamsters (*14*).

Other authors, however, reported an increased incidence of experimentally induced lung tumors in hamsters fed a diet containing high vitamin A levels (*20*). In this respect interesting recent findings in ultrastructural studies performed at our institute are worth mentioning. Hamsters given a high vitamin A diet (400,000 IU/kg diet) showed a considerable increase in size and number of lipid droplets in alveolar fibroblasts. Similar droplets have been found in alveolar walls and in the interstitium of the renal medullar of rats after feeding varying levels of vitamin A (*21*). In these droplets vitamin A was observed. In droplets closely resembling those seen in the interstitial fibroblasts of the renal medullar of rats and rabbits arachidonic acid was found (*22, 23*). This acid is a precursor for the synthesis of prostaglandins and thromboxanes. Since both substances can be synthesized in the lung (*24*), it may be speculated that the droplets found in the lung fibroblast as a result of a high vitamin A intake will contain arachidonic acid and may therefore be of importance in the synthesis of prostaglandins in the lung. It has been postulated that prostaglandins play a role in carcinogenesis and this may be a possible mechanism in which vitamin A exerts its action in respiratory tract carcinogenesis.

Possible Carcinogenic, Cocarcinogenic and/or Synergistic Properties of Six Cigarette Smoke Components

Cigarette smoke is composed of hundreds of gaseous, liquid or solid components (*1*), from which only a relatively small part has been examined for carcinogenic or cocarcinogenic properties. The number and potency of known cocarcinogens in cigarette smoke, however can not explain the high frequency of lung tumors in man as a result of smoking habits. The carcinogenic action of cigarette smoke will mainly depend on cumulative, synergistic and antagonistic action of the different components on the respiratory tract.

A cigarette smoke component that possesses remarkable comutagenic properties is norharman. This component is not mutagenic itself, but it can considerably enhance the mutagenicity of two carcinogens, B(a)P and H-2-fluoranylacetamide (*25, 26*). In addition, the carcinogen Yellow OB alone is not mutagenic, but in combination with norharman it clearly induced mutagenicity (*27, 28*). Norharman has not appeared to be a carcinogen after chronic oral intake, neither did norharman show a cocarcinogenic action when fed together with aniline to rats (*29*). The action of norharman on the respiratory tract is, however, unknown.

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